Facsimile Cover Sheet

To: Examiner Zeman
Art Unit 1815
Company: U.S. Patent & Trademark Office
Phone: 703-305-7133
Fax: 703-305-7401

From: Heidi S. Nebel
Company: Zarley, McKee, Thomte, Voorhees & Sease
Phone: 515-288-3667
Fax: 515-288-1338

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Pages including this cover page: 14

Comments:

08/739,264

William E. Marshall, et al.

METHODS AND COMPOSITIONS FOR MODULATING IMMUNE SYSTEMS OF ANIMALS

OFFICIAL

Rec'd 4/23/98 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT	MARSHALL, et al.	ART UNIT:	1815
SERIAL NO:	**08/739,264**	EXAMINER:	M. Knode
FILED:	**October 29, 1996**		
TITLE:	**METHODS AND COMPOSITIONS FOR MODULATING IMMUNE SYSTEMS OF ANIMALS**		

#9
KD 4-27-98

TRANSMITTAL OF RULE 132 DECLARATION

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Attached herewith is a § 132 declaration of the inventor Dr. William E. Marshall of the above-identified application. It describes experimental results which further substantiate and support the earlier amendment filed on January 12, 1998.

It is respectfully requested that this declaration be considered and made of record in the above identified case.

Respectfully submitted,

Heidi S. Nebel,
Reg. No. 37,719
ZARLEY, McKEE, THOMTE, VOORHEES & SEASE
ATTORNEYS OF RECORD

801 Grand - Suite 3200
Des Moines, Iowa 50309-2721
515-288-3667 - pw

CERTIFICATE OF MAILING (37 C.F.R. § 1.6(d))

I hereby certify that this § 132 Declaration is being transmitted via facsimile on the date shown below to the Assistant Commissioner of Patents, Washington, D.C. 20231, attention Examiner Mary Zeman---(703)305-7939.

4/23/98
Date

Heidi S. Nebel

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

recd 4/23/98
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SERIAL NO:	08/739,264	EXAMINER:	M. Knode
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132 DECLARATION OF DR. WILLIAM E. MARSHALL

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Dr. William E. Marshall hereby declare the following.

1. I am the inventor on the above-identified case and am familiar with the prosecution including the office action dated July 10, 1997.

2. My background includes a Ph.D. in biochemistry from the University of Illinois, post-doctoral training at Uppsala University and Cambridge University, assistant professor of biochemistry at the University of Minnesota, director of technology development at General Foods Corp., president of the Microbial Genetics Division of Pioneer Hi-Bred International, member of the Iowa Academy of Sciences, chairman of the National Agricultural Research and Extension Users Advisory Board of the U.S. Congress, member of the advisory panel on biotechnology to the Office of Technology Assessment of the U.S. Congress, member of the advisory panel on intellectual property to the GATT, and associate professor of microbiology and immunology at the New York Medical College.

3. This declaration brings forward evidence of the numerous ways that we have stressed bacteria to induce the production of stress response factors.

4. The term "stress" as it relates to microorganisms particularly with respect to chemical, physical or biological stress, is a term known and understood to those of skill in the art of microbiology. One recent definition of stress is found in Microbiological Reviews 59:(3), 506-531 (1995), "Stress-Inducted Transcriptional Activation" by Willem H. Mager and Adriaan J.J. De Kruijff. "Living cells display a rapid molecular response when they are exposed to adverse environmental conditions. This ubiquitous phenomenon is commonly designated stress response, and it can be considered a general reaction to metabolic disturbances."

5. Based upon my years of research with stress response factors, we have shown, and it is my opinion, that any form of stress for bacteria causes release of stress response factors.

6. I have personally been involved in experiments in inducing stress to bacteria by numerous means using chemical, biological or physical stress known to the art of microbiology.

7. My goal has been to understand the relationship between bacterial stress and the host immune system. My experiments have focused on those adverse environmental conditions that were commonly being encountered by bacteria either during ingestion by animals or as part of the normal flora populating the non-sterile tissues of animals (i.e. the oral nasal cavity, the outer ear, esophagus, stomach, intestinal tract and vagina). Transferring from culture to saliva, nutrient deprivation, concentrating, diluting, heating, and exposure to antibiotics would all be examples of typical stresses encountered by these bacteria.

8. I have personally been involved in experiments conducted to evaluate the following stress conditions and their ability to generate SRP's as listed below.

(a) Do bacteria release SRFs at the same rate when transferred into either 0.01M phosphate buffer, phosphate-buffered saline, or Minimal Media-Davis plus 0.1% dextrose?
Yes. Notebook V, page 1, July 15, 1996.

(b) Do bacteria release SRFs from their stationary phase as well as from their log phase?
Yes. Notebook V, pages 3-4, July 17, 1996.

(c) Are SRFs released after exposure to antibiotics?
Yes. Notebook V, pages 14-15, 18-22, and 44-46, July 30, 1996.

(d) At what dilutions will bacteria begin to release SRFs?
A 10% dilution of the culture will induce the release of SRFs at a level equal to 80% of that released in 100% non-nutritive buffer. Notebook V, pages 23-27, August 5, 1996.

(e) At what concentration of crowding do bacteria begin to release SRFs?
When cultures are concentrated by 3-fold, SRFs are released at a level lower than that released at 10-fold concentrations. Notebook V, pages 25-27 and 75, August 6, 1996.

(f) Do whole plant corn silages release SRFs when transferred into non-nutrient environments?
Yes. Notebook V, pages 30-31, 35-38, September 9, 1996.

(g) Do silage inoculant strains (e.g. *L. plantarum* and *E. faecium*) release SRFs when transferred from broth to 0.1M acetate buffer, pH 4.0?
Yes, but at a level lower than at pH at or above 6.5. Notebook V, pages 39-43, September 25, 1996.

(h) Do silage inoculant strains release SRFs when transferred from broth to saliva?
Yes. Notebook V, pages 39-41, September 25, 1996 and Notebook VI, pages 1-27, April 2, 1997.

(i) Do milk strains (*L. caseii*) release SRFs when stressed in 0.9% saline, saliva or phosphate buffers?
Yes. Notebook V, pages 69-73, December 10, 1996.

(j) Do yogurt strains release SRFs when transferred from broth to saliva-mimicking buffers?
Yes. Notebook II, pages 36-40, January 10, 1995.

(k) Does X-ray irradiation induce the release of bacterial SRFs?
Yes. Notebook I, pages 20 and 25, May 26, 1993.

(l) How rapidly are SRFs released?
SRFs are released within the first 10 minutes when transferred from culture to phosphate-buffered saline, pH 7.6. Notebook VII, pages 45 and beyond, July 21, 1997-April, 1998.

9. All of the above-identified experiments were conducted using standard experimental conditions and using a protocol similar to that evidenced by the notebook pages attached herewith which evidence stress response factor generation after exposure to antibiotics (c) and to saliva mimicking buffers (j).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Date: Apr 23, 1998

William E. Marshall
William E. Marshall

[redacted]

Yogurt = plated on MRS; 1000 taken Wed 5^{30}_{p} 1-4-95 in 1ml DI. 10ml added p36

Mon 1-10-95

Grow 649 & 2362 @37°C stat for 3-4 days.
Single colony from yogurt " "

@ 11^{30}_{a} A_{570} aq H_2O

649 Broth -.109 0.981 .890 .872 returned to 37°C stat

2362 Broth 0.553 | .444

Yog MRS. = 0.726 not diln. mixed - .400

20 ml MRS added + placed in 35 ml cent tube mon 11^{30} at 37° stat

TUES @ 11^{30} 24 Hr later

649 • 1.407 aq H_2O -.109 = 1.3

2362 .588 " -.109 = 0.479

Mon 1/16 Loop

649 1.353 -.109

2362 1.144 -.109

YOGURT 2.516 -.400

TUES 3.5 8

5ml" 8830 1+9 1.728 filtered" placed in dial bag 6-8 and placed in 10ml H_2O in bag <3.5 + 3000ml Saline @4°C

4^{00}_{p} IV Mouse g ml B8

Fri 2 x 4 mice Balb/c inj 400ug LPS B8 & Alex

Sat 6^{10}_{p} 1 mouse in each group dead. } 26 Hrs

Sun 12^{00}_{n} 3 live mice in 10% Group
1 live mouse in 1% Grp. } 40 Hrs

Mon 1^{00}_{p} 3 live mice in 10%
0 live mice in 1% } 69 Hrs.

Re

A
B

Sun

smells

Smells
contam

C

smelled

6/11/00 (?) 166

see p18 - file 317

Recap of last 2 mouse trials

LPS 400 ug 0111:B8 ip. to Balb/c ♀

4 mice per group

Control (1/6 x Isotonic Saline =) 0.15% NaCl; 0/4 Survived

(A) Treatment 10% (i.e. 15ml fermented lactic in 135ml H_2O) 3/4 Survived

(B) 1% (i.e. 1.5ml " 149.5) 1/4 Survived

	Control	10%	1%
Survival	0%	75%	25%

? MSG

LPS on perit. mφ ≥ oroph Mφ

Control - H_2O; 0/4 Survived in 48 Hrs ; [F] 5 day 68ml

smells S-contain. 2% 0.15% NaCl; 0/4 Survived in 48 hrs; " " 68ml

1% 1/4 Survived " ; " 3 day

10% 3/4 Survived 3 days

smells S-contain. ~~12% 0/4 Survived " 5 days~~

Control 0.15% NaCl 2/4 Survived " 5 days

All Received 400ug ip of 0127:B8 (12-29-94)

	Consumption			(Survival) Recept 48h
H_2O				0/4
0.15% NaCl	62 ml consumed, 5 days			2/4
1% in H_2O (0% NaCl)		3 days	#x10^7	1/4
10% in H_2O (0.1% NaCl)		3 days	#x10^8	3/4
smelled of S-contain. 15.5%	68 ml consumed, 5 days		#x10^9	2/4

ee
ul
o stat
1/16
.53
.09
.14
.9
2.516
.400
/c
ley

p38

Mm 1-16

Lm 649 in Saline
Lm 2362 "
Yogurt "Y. #1" "

A_{770}
1.794 in 7. ml
1.681 in 7. ml
2.558 in 10. ml
↓
1.823 in 70 ml

A_{770}	A_{660}
1.794	1.589
1.687	1.462
1.823	1.727

37° Stat #/4
"
5 in 2 fuel centrifuge
Stat
Shaker
Mm @ 5^{00} p

M81 { A_{550} = 2.307 in 168. ml → 90
A_{660} = 2.241

20. ml 1/5
4 → 20 1/10
2 - 20

Shaker
2 to 20 1/10
shaker
4 to 2 p M81 1/100

Shaker a 20. ml
b 1.68 to 17. ~~2 to 18~~
c .34 to 17. ~~.4 to 19.6~~

A_{770}	A_{660}
2.307	2.241

in 168. ml of Saline

spun down the pellet & ~~168 ml~~ taken up in 20. ml of saline

ⓐ "as is" - i.e. 20. ml "[A_{550}] × $\left(\frac{168}{20}\right)$

ⓑ 1.68 ml of ⓐ + saline to 17. ml - 1:10

ⓒ 0.34 " " " - 1:50

placed on shaker Mm @ 5^{00} p

Contr

TUES ~~Weds~~ @ 4^{50}

Mon @ 4^{00} 30 ml left in 1% = 148   148 - 30 = 118

Fri @ 4^{00} 40 " "   148 - 40 = 108

108 ml drunk over ~~4~~ 3 days before ip. injection or $\frac{27}{4}$ ml per mouse per day (6.75 ml)

Mon @ 4^{00} 70 ml left in 10% = 150 - 70 = 80 consumed

Fri @ 4^{00} 8?

Wi
Lm
Lm
Yc
Lp
Lp
Lp
ha
d
15
Swi
10^{00} a

39

of cc 48 Hrs < Shaker / Stat .43 Hrs

WED @ 4 ^{20} p

		A_{550}	A_{660}
Lm 649 (Stat)	(-2×10^9)	1.678	1.377
Lm 2362 (Stat)	(2×10^9)	1.559	1.272
Yog " Y #1 " (Stat)	(2×10^9)	1.796	1.686
" (Shaker)	(2×10^9) 48Hrs:	1.821	1.720
LpM81 "10:1 As Is" (SHAKER)	10x $(\sim 7 \times 10^{10})$	8.4×10^{10} too dense	
LpM81 " 1:10 or 2.307 x SHAKER	$(\sim 7 \times 10^9)$	7.4×10^9 2.154	2.083
LpM81 " 1:50 or 1.5x SHAKER	$(\sim 1 \times 10^9)$	1.5×10^9 1.222	1.003

3rd BB Mouse Study - TUES 1-18-95

la/lc { Repeat 1% 148.5ml A/c H_2O + 1.5ml la + lc

dupl. { 10% 135. ml A/c H_2O + 15. ml La + Lc

10% 135 +15 ml (S)

Control 10% A/C Saline (150?)

15g each 5wks old ♀ 10% 8830 11 ml 8830 + 100ml A/c H_2O

Wed 1-19-95 all mice - OK. * control mice H_2O adjusted To 100ml @ 1^{45} p today

Thur 1-20 " OK | 24 Hr Consumption

10^{00} a Fri 1-21 all mice OK

See p20 of Record Book *

"Yogy#1" p40

"Yogy#1" 2.558 in 10 ml

↓ dil to 70 ml

1.823/1.727 550/660

35 ml Stat

6½ ml taken after

24 Hrs

48 Hrs 1.796/1.686

72 Hrs

~~144 Hrs~~ 120

35 ml Shake

24 Hrs TUE

48 Hrs WED 1.821/1.720

72 Hrs Thurs

~~144 Hrs~~ 48 120 Sat 2ti 1/21 Sun

on Fri 1/20 Cent 48 Hrs incub for testing - hold the Rest

Resuspend pellets = Same volume for 2nd Rel of 48 Hrs

Compare Shake vs stationary after 48 Hrs. first hold the rest in pellets

① Spin "10" Lp 7.4 x 10^{10} → FDCS

② Restart Lp MS1 7.4 x 10^{10} for 2nd Rel of 48 Hrs - STAT on Sat @ 6^{00} p

③ Sun stop Yog Shake / Stat

(B) Are SRPs Released when P/s is added to ~~log~~ stat Φ ?
15 ml gears

1177S from previous preparations preps in MMSD + .1% - final 3/4 hr on shaker

A_{540} before shaker = 0.393
A_{540} ~~before~~ before P/s addn = 0.559 CFUs = $\frac{23+25}{2}\times10^{7}$ = 2.4
A_{540} 3 Hrs ~~after P/s addn~~, no P/s = .765 CFUs = 6x10^8
A_{540} " after P/s added = .665 <1x10^7

9:00 CFUs @ the time P/s was added = 2.4x10^8
12:00 CFUs after 3 Hr exposure to P/s = $\frac{0+0}{2}\times10^7$

3 H, no P/s → 6x
3 H control → $\frac{54+67}{2}\times10$ → 6x10^8

Blank = MMSD + .1% unused + 1% P/s

Read vs H_2O	No P/s	P/s added (.15 to 15 ml)	P/s in H_2O (in 10 ml H_2O)	
220	2.630	2.720	0.738	
230	1.780	1.936	0.214	
240	0.910	1.159	0.046	
245	0.656	.921	- 0.015	
250	0.481	.741	- .080	.422
254	0.379	.628	-.130	.357
260	0.251	.485	-.191	.276
265	0.166	.368	-.231	
270	0.077	.235	-.272	
275	-0.010	.100	-.297	
280	-0.090	-.026	> -.301	-.111
	↓ thru 10	↓ thru Amicon 10	↓ thru Amicon 10	

≥ 95% killed in 3 Hrs

A_{220}
240
250
254
260
270
280

SAVE

not read

G-10 on p18

concen roto-vap yellow ~~ppt~~ c̄ ppt amt of ppt - spun off

→ G-10 p18

Save

Left margin: [illegible]; 12; 60 ml; - 29 ml; read vs H_2O; Control; [illegible]; conc

[redacted]

15

7-30-96 TUE

Are SEB's rel. in Stat Φ after P/s treatment?

2x 15ml propagation from p14 allowed to propagate into their stat Φ's (by standing O/N @37°C). To one was added 0.15ml P/s (A 1%) & allowed to stand 24 Hr @ 37°C.

MMD 1.1% aq. H_2O @ 540 = ref = 0

VERY LITTLE INHIBITION OF Ec BY P/s O/N

	+ P/s .657	no P/s = ~~.705~~	P/s Std Blank (10% in [illegible])
A_{540} =	.657	.705	
CFUs @ 10^{-7}	$\frac{42+43}{2} \times 10^7 = 4 \times 10^8$ (F)	$\frac{62+70}{2} = 6.5 \times 10^8$	
1 A_{220}	1.343	.962 (all 3" read ag MMD +.1%)	.866
11 230	.938	.624	.615
21 240	.788	.472	.475
26 245	.752		
31 250	.718	.461	.358
35 254	.689	.470 PEAK	.315
41 260	.637	.473	.261
51 270	.485	.413	.188
61 280	.311	.290	.143

↓ Amicon "10" ↓

A_{220}	1.088	.979	.661
230	.818	.664	.493
240	.699	.530	[illegible]
~~25~~ 250	.622	.501	[illegible]
254	.593	.500	.268
260	.545	.490	.235
270	.414	.462	.171
280	.267	.280	.114

↓ conc Rotovap ↓

G-10	G-10
p19	p21

7-31-96

G-10 of <10 kDa fract. ref. of P/s-treated Ec for 3H in log ϕ (see p14). new col. - 1st use

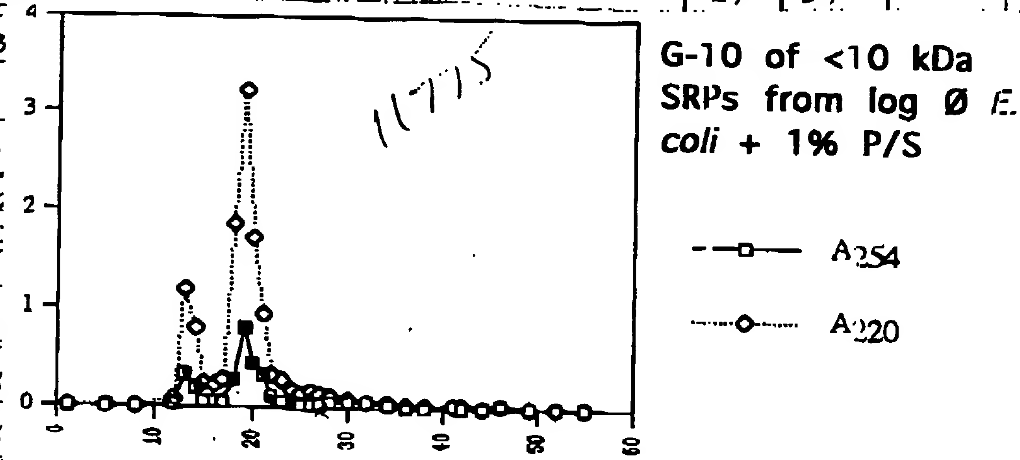
.01M PO_4

all swadded

P/s inhibited growth

18

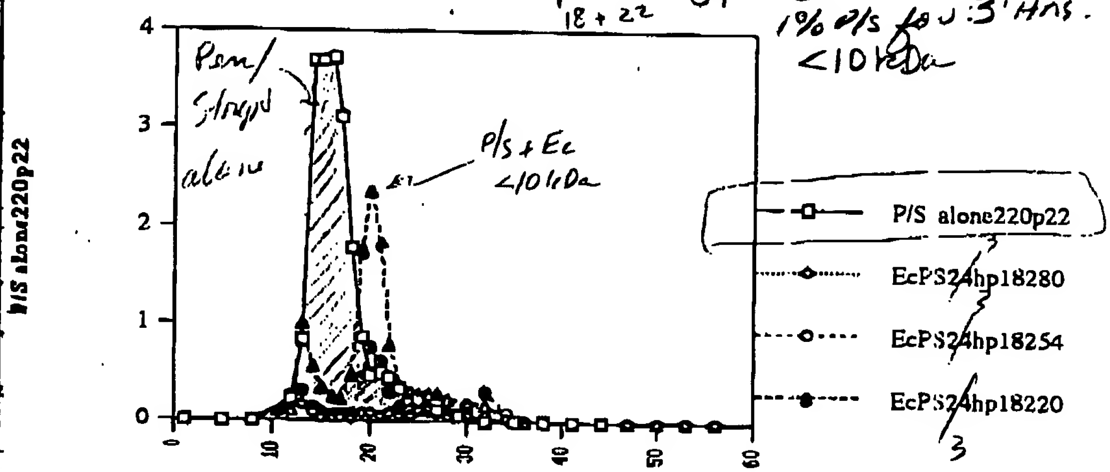
fr	e/v	A_{220}	A_{254}	A_{280}		fr	e/v	220	254	280
1	7.0					29	37			
2	1.3 / 8.3								.013	-.029
3	9.6									
4	10.9									
5	12.?								.005	.014
6	13.5								0	-.013
7	14.8									
8	15.8								.013	-.010
9	16.8									
10	17.8	1.846	.254	.074	Ib 11 kd	0	6	.013	.010	.011
1	19	3.2	.785	.170		9	7			
2	20	1.696	.421	.050		40	8	~~.017~~	~~.020~~	~~.020~~
3	1	.922	.308	.049		1	9	.017	.020	.020
4	2	.310	.106	.024	II 3H	2	50			
15	3	.261	.043	.004		3	1			
6	4	.156	.027	.008	(i.e.	4	2	.031	.032	.034
7										
8										
9										-.027
20										
1										
2										
3										
4										
25										
6	7	.057								
7	5					5	3			
8	26	.009	-.005	.025		50	4			



G-10 new

p14, log ϕ Ec 11775 treated c 1% P/s for 3 Hrs. <10 kDa

18 + 22



all zero out to 7?

STOP